Chapter 2

LABORATORY INFRASTRUCTURES

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Introduction

Plant tissue culture is a popular technique to reproduce plant species that are rare, endangered, medicinally and economically important species. This technique is also helpful for these plants, who does not propagate by conventional methods as seed or vegetative means. This way of plant propagation offers a lot of distinctive advantages over conventional propagation practices in the sense of rapid multiplication, non-seasonal production and prompt release of value-added varieties at commercial scale. To become financially viable, any tissue culture project must operate at optimal level of production. For optimal production, tissue culture laboratory should be intended after taking into consideration the entire accessible infrastructure and should be utilized to their full competence. The laboratory infrastructure is a main factor affecting the productivity as well as financial feasibility of the any tissue culture project. Basic infrastructure of tissue culture laboratory mainly comprises of the space or area for different deeds (i.e. media preparation and sterilization, washing and storage of labwares, aseptic manipulations, maintenance of growth cultures etc.), equipment's (i.e. autoclave, laminar air flow, hot air oven, weighing balance, pH meters, glasswares etc.) and chemical requirements (organic and inorganic nutrients, growth hormones, gelling agents etc.). The floor plan of the laboratory is designated to maintenance of the temperature, illumination, as well as ventilation and it should promote high standard of work. Besides, careful planning is required while considering the size and location of laboratory to availing of basic facilities (natural lighting, electrical

or water supply, drainage etc).However the size and infrastructure of the laboratory has been decided by the nature of the project undertaken and the availability of the funds for this purpose. For successful operation of a large or small tissue culture laboratory, distinctive infrastructure will not only help to maintain aseptic conditions, but will also achieve optimum level of production. Thus, this chapter summarises the essential infrastructure or facilities that are required for the successful tissue culture operations. Furthermore, safety considerations in the tissue culture laboratory are also described.

Various fields in which tissue culture techniques are used, shown in Fig.1

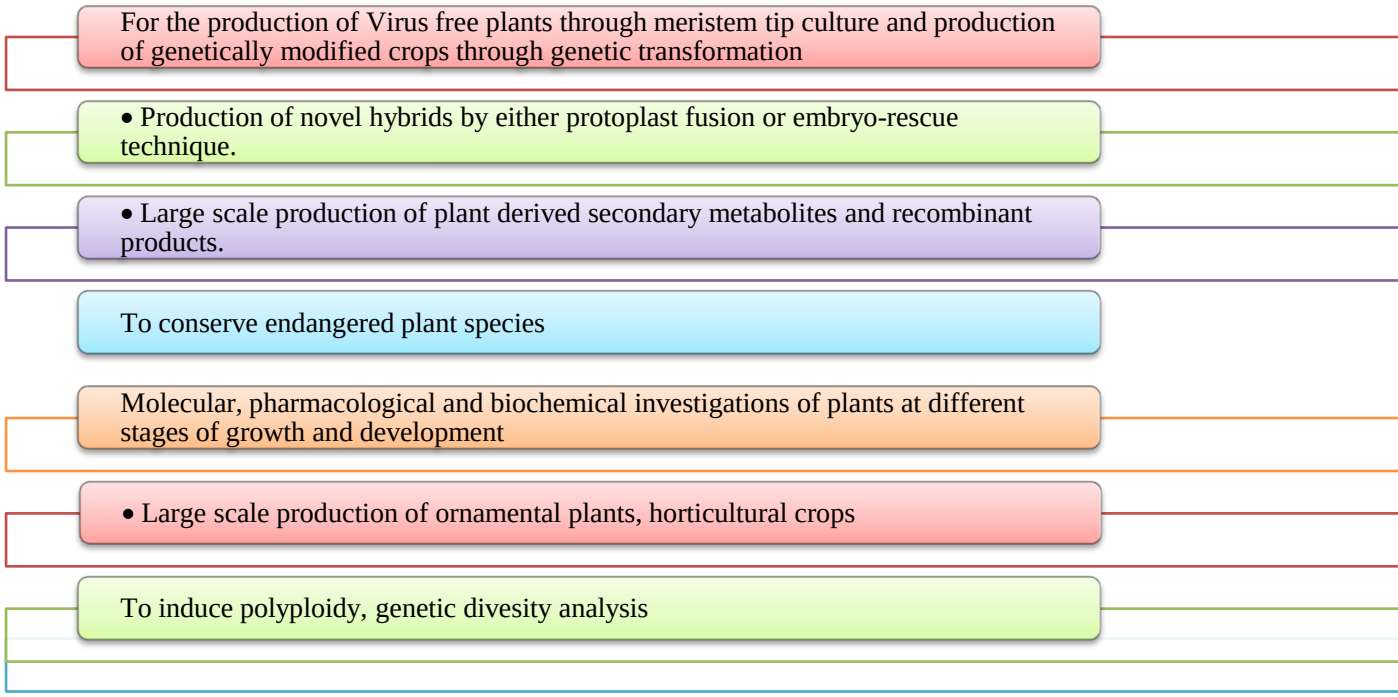
For the production of Virus free plants through meristem tip culture and production of genetically modified crops through genetic transformation
• Production of novel hybrids by either protoplast fusion or embryo-rescue technique.
• Large scale production of plant derived secondary metabolites and recombinant products.
To conserve endangered plant species
Molecular, pharmacological and biochemical investigations of plants at different stages of growth and development
• Large scale production of ornamental plants, horticultural crops
To induce polyploidy, genetic divesity analysis

Fig 1: Uses of Plant tissue culture techniques

2. Types of Tissue Culture Laboratory

On the basis of the production capacity, the tissue culture laboratories may be divided into two major categories including: (1) small scale laboratory and (2) large or commercial scale laboratory. A small-scale laboratory is basically used for the specific research and development pursuits. While in large scale

laboratory commercial scale production is done. Mostly laboratories (research or commercial labs) are set-up in already existing structures, only few can construct from the ground up. In this case some basic guidelines should be followed. The location of newly constructed laboratory should be away from the city /crowded area or otherwise facility should be protected from pollution and vibrations of vehicles. The proper precautions should be taken from heavy wind and rain because these are carriers of microbes, spores, and insects etc .The small scale laboratory requires minimum area for washing, media preparation, transfer as well as growth room and estimated area for this purpose is about 200 square feet. With basic equipments, chemicals and proper water or electric supply the laboratory can start their functioning. While in large or commercial scale laboratory the area of washing, media preparation, and transfer as well as growth room decided according to the installed capacity and it can be divided into separate rooms. The basic infrastructure facilities are similar to the small scale laboratory but their quantity can be depends on installed production capacity of the laboratory.

3. Basic Infrastructure for Tissue Culture laboratory

Despite the difference among the production requirements, basic infrastructure facilities are common in both the small and large scale laboratories. The basic infrastructure for a tissue culture laboratory is mainly described in following major sections including :(1) structural areas or places (2) equipments, (3) chemicals, and (4) other requirements. Adequate water supply is also required in the tissue culture facility and laboratory should be away from local municipal waste/public water or sewer facilities. Disposal of water should be eco-friendly according to local municipal rules. Emergency power supply is also required to prevent risk of experiment ruin, even transfer room, culture room and essential instruments must be attached to generator back up facility. Size and design

of laboratory depends on shape and size of land available and the capacity of company.

3.1 Structural areas or places

The ideal tissue culture laboratory provides following structural facilities i.e. nutrient media preparation and sterilization, washing room, aseptic manipulation or culture transfer room, culture room with controlled environment conditions, storage room and acclimatization room. These structures can be changed according to the available space, installed capacity and the available financial source for the laboratory. A clear cut demarcation between clean area (transfer room and culture room) and unclean area (washing area, media preparation and autoclaving area, office, storage room etc) should be maintained in the laboratory for good quality work. General entry into clean area should be restricted. Restricted entry into this area provides aseptic environment. When researchers enter into this area must change outer cloths, take off slippers or shoes, wash hands and foot, and wear mask, gloves, slippers which are provided inside the laboratory. Positive air pressure should be maintained in clean area. These precautions are essential to maintain aseptic environment. Well planned infrastructure of room and direction of windows, doors and corridors may provide maximum production and cleanliness. The various work areas should be inter-connected for frequent movement of material and easy access. A systematic blue print of a laboratory infrastructure is provided by the authors.(Fig 2)

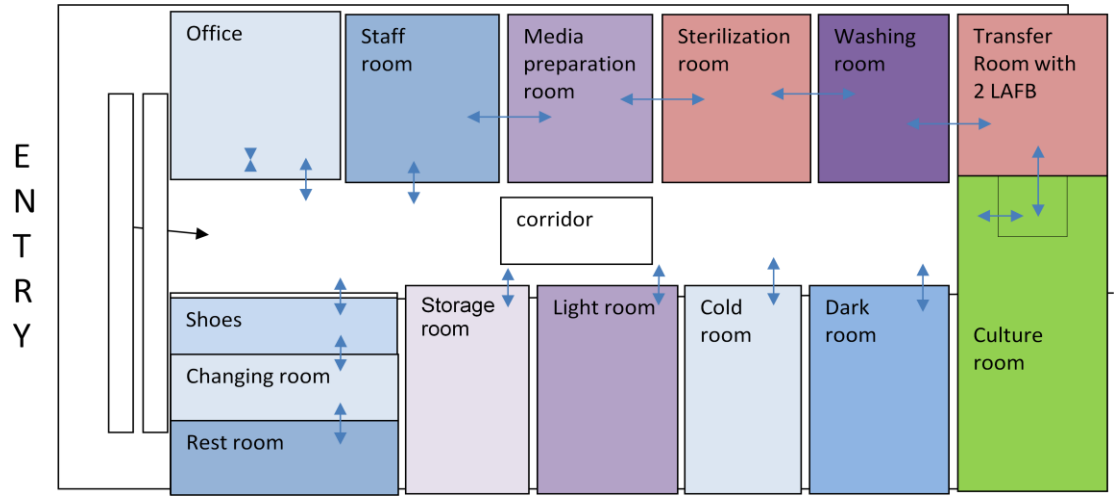

Figure 2: A blue print of a laboratory infrastructure. Office (A). Staff room (B).Media preparation room (C).Sterilization room with autoclave (D).Washing room with drying oven and distillation unit (E).Transfer room with two LAFB (F).Culture room with controlled conditions of temperature, light, humidity etc. (G) .Dark room, cold room, and light room for specially designed culture growth (H). Storage room for storing glasswares/labwares, chemicals and instruments.(K).Changing room which contains masks, gloves, shoes and sanitizers (L).Rest room (M).

3.1.1 Media preparation and sterilization room

The media preparation and sterilization room is an important part of the laboratory where most of the activities are performed. This room should have sufficient space for store the frequently used chemicals, glasswares and instruments which are required for the media preparation, sterilization and storage. The location of media preparation and sterilization room should be well planned such a way that it assure the frequent movement of glasswares, water and other essentials. Following facilities are required for preparation and sterilization of media.:(a) The working benches should have 3 to 3.5 feet height and about 6 x 4 feet width for comfortable working and handling.(b)deep freezer to store stock solutions, enzymes, coconut milk

etc.(c)refrigerator for storage of plant material, short term used chemicals and solutions.(d) plastic cans for distilled water storage (e) weighing machine (f) pH meter (g) magnetic stirrer (h) membrane filtration for thermolabile chemicals.(i) microwave or steamer to melt agar or media.(j)autoclave or pressure cooker for sterilization of media and glasswares. Weighing balance should kept into dry chamber in the media room. A deep freezer or refrigerator may be kept out side the media room. Proper water supply must for media room. A good quality water is mandatory for preparation of media, tap water not to be used. In the teaching laboratories de-ionized water may be used. While in the research or commercial scale laboratories milli-Q water purification unit or water distillation unit must be installed. Milli-Q water free from microorganisms, particle traces, colloids, ions, organic impurities etc. Whereas de-ionized water free from ionic impurities. Prior to the culture media preparation glasswares, culture vessels, equipments etc should be sterilized. The prepared culture medium may also provide favourable environment for microbial growth, because medium is a richest sources of nutrients. To avoid this contamination culture media must be autoclaved at 121˚C temperature for 15-40 minutes. If autoclave facility not available then pressure cooker may be used. On the basis of the volume of the culture medium, sterilization time may be varies (see Table: 1)

S. no.	Volume of media (ml)	Sterilization time at 121˚C(minute)
1.	20-50	15
2.	75	20
3.	250-500	25
4.	1000	30
5.	1500	35
6.	2000	40

Table 1: Relationship between Sterilization time and media volume (Reconstruct from S.S. Bhojwani, M.K. Razdan. Plant tissue culture: Theory and practice)

Some authors reported that high temperature (120˚C) exposure decreases the nutritive value of media, autoclave at 110˚C temperature for 20 minute provides best results for media sterilization. The thermolabile compounds (e.g. plant hormones, plant extracts, vitamins, proteins and enzymes etc) used in tissue culture should not be autoclaved. A membrane filtration is used for sterilization of these compounds. Membrane filter have bacterial proof membrane with pore size 0.45 µm or less, which is adequate to prevent microbial contaminants. In media preparation and sterilization process, a lot of steam, heat, and moisture is generated. Thus, this area must require at least one exhaust fan and one window to remove the warm and moist air to protect walls and floors of laboratory.

3.1.2 Washing room

The washing room is largely used for labwares cleaning and washing, which are used in tissue culture experiments. The washing room should contain a large sink, brushes of different shape and size, hot and cold tap water, distilled and double distilled water, detergents etc. The sink mainly made up of lead lined, which is resistant for acidic and basic solutions. It will also require adequate space on both sides of the sink for soaking and drying of the glassware or plastic ware. Plastic netting should be required near the surface of sink to prevent the breakage of glasswares. The glasswares washing, drying, and storing, a large plastic or steel bucket to soak the labwares, hot air oven and dust-proof cupboard is also required respectively. After the proper washing and drying, glasswares should be placed in dust-proof cup board or air-tight storage room to prevent the destruction caused by dust particles and allow easy access. Washing room and media preparation and sterilization room should be inter-connected to each other. Most of laboratories have these both facilities in a single room. In this

situation temporary separation should be carried out between washing area and media preparation area. If this is not possible, then timing of media preparation and washing should be so arranged so it does not interrupt each other.

3.1.3 Aseptic manipulation or Culture transfer room

The culture transfer or aseptic manipulation room needs special attention. Hence this room needs to be minimum air disturbance and high aseptic environment. To achieve aseptic environment, most of the laboratories are installed laminar air flow cabinets for transfer cultures.(See fig. no.3 Culture transfer room with laminar air flow cabinet). In temperate areas laminar air flow cabinets are kept in normal laboratory. However in tropical and sub tropical areas the cabinets should be fitted in separate room with double door facility, which maintain aseptic environment and also prevent from atmospheric dust. In the culture transfer facility, spatulas, needles, scalpels, forceps etc are required for aseptic manipulations. During the aseptic manipulation or the transfer of cultures, these equipments must be sterilized by 70-95% ethyl alcohol and then flaming and cooling. This process should be carried out several times during tissue culture operations. Bunsen burner is used for flaming of the equipments. However, Bunsen burner have some disadvantage, even minor mistake may take fire hazards or cause heat burns. Recently glass bead sterilizer and infra-red sterilizer are used for sterilization of these instruments. These sterilizer have well insulated body and regulator, for minimum exposure of heat and regulate temperature respectively. However, this room have no need of special requirement (i.e. specific light, temperature etc.) but their walls and floors must require frequent cleaning. Special laboratory shoes or slippers, mask, cap and aprons should be worn in this area, to decreases the circulation of contaminants by a personnel. This room should be connected with generator facility.

Figure 3: Transfer room with laminar air flow cabinet

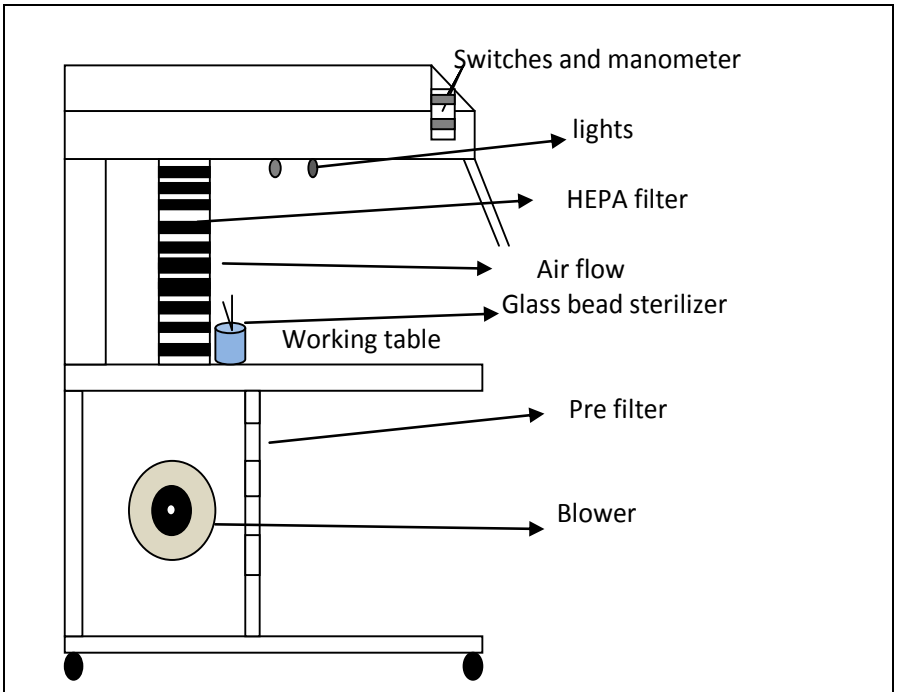

Figure no. 4:-Schematic diagram of horizontal LAF bench

3.1.4 Culture or incubation room

The culture room or incubation room plays a substantial role in the successful operation of tissue culture laboratory. The culture or incubation room has controlled environmental conditions of temperature, humidity,

and light. Air circulation should also maintain for optimal growth. Temperature plays a pivotal role in culture room. The temperature used in the incubation room maintained around 20-30 ˚C. To achieve this temperature air conditioner and heaters can be used. For special temperature treatment (lower or higher temperature) special incubators are also used. These temperature controller units should be placed outside the room or in the corridor with adequate precautions. Light used in culture room most probably diffused light (less then 1klx). However some experiments require high light intensity (5-10klx) or total darkness for culture growth. Automatic time clocker should be used to controlled temperature and light. Relative humidity should be maintain below 50%, which is optimum for growth and also prevent culture media from drying rapidly. If relative humidity higher than 50% can cause damping of cotton plugs and increase risk of contamination in the culture. The cultures are stored in the specially designed culture racks or shelves in the culture room (See Fig 5.Culture shelves with cultures). The culture shelves may be either fitted on to culture room walls or iron frames, which are placed in the room. The culture shelves are constructed from rigid wire mesh or glass. Each shelf has a separate set of fluorescent tube lights. Insulation should be maintained between fluorescent tubes and shelf to prevent intensity of hot air and assure highly uniform temperature around the whole cultures. Ventilation should be maintained in culture shelves. A small fan is fitted at one end of the shelf and hollow pipe with holes at convenient distance running the length of shelf. Which allow more even air flow around the culture.

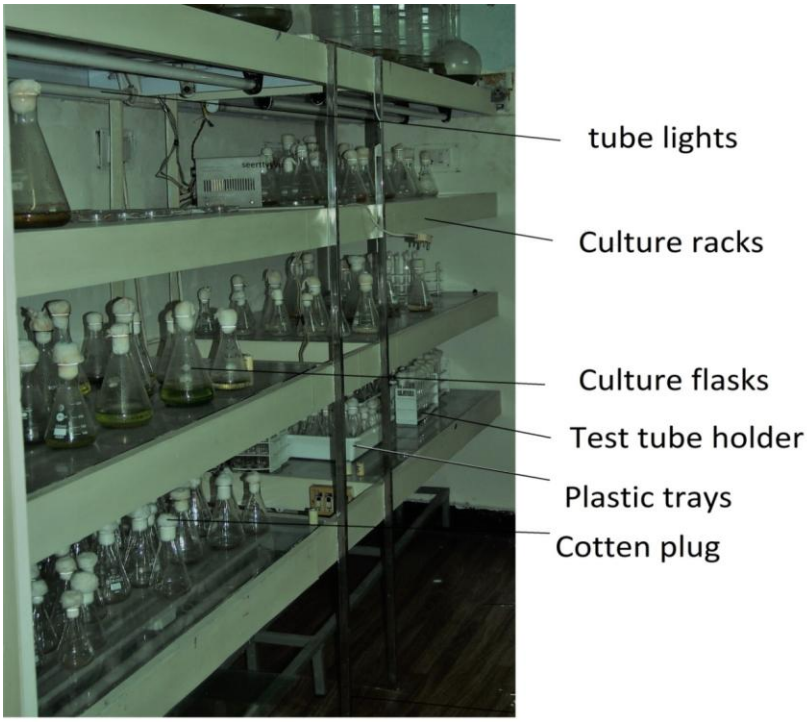

Figure 5: Culture shelves with cultures

In most of laboratory culture flasks, jars, petri-plates are placed directly on the shelf, while culture tubes placed on metallic wire racks for support. Which have holding capacity of 20-24 tubes. Experimental details (such as name of plant and explants, date of experiment, media, name of researcher) should be labelled on culture tubes or trays. Shaker machine should be installed if suspension cultures are grown in the culture room. It may be horizontal or rotatory type with controlled temperature and light are also available. Walls and floors of the room must require frequent cleaning and joints of walls should be rounded rather than angular to avoid cob webs. Generator facility is mandatory for this room. The commercial laboratories may have separate growth rooms for different growth conditions such as dark room, cold room and light room is required for specially designed experiments.

3.1.5 Storage room

A variety of glasswares, plasticwares and labwares are stored in this room. Chemicals and instruments which are not frequently used also stored in this room. This room not need to be special requirements (i.e. temperature, light etc.) but their walls and floors require frequent cleaning.

3.1.6 Acclimatization area or green house chamber

In tissue culture laboratory, acclimatization area or green house chamber is required primarily for the hardening of plantlets in natural environment and maintaining of the mother plants for future production cycles. This area, have minimum facilities for maintaining high humidity, low light, cool environment for summers and hot environment for winters must be provided. The hardening chamber needs high humidity (90-100%, by mist and fog systems) and high illumination (4,000-10,000lux). Humidity is required for establishment of tissue culture plants after taken out from rooting media and transfer to pots under greenhouse. A green house is a structure with walls and roof made primarily of transparent material (such as float glass, plastic films, fiber-glass reinforced plastic and acrylic as well as polycarbonate panels) which permits natural light (Sun light) for the plant growth. According to their size and construction greenhouse can be categorized in four major types including the Tunnel Type (height up to 2 meter, constructed by bending bamboo or steel tubes covered with polyethylene), Ground-to-Ground Type (height at the centre up to 4 meters, constructed by mainly steel tubes covered with high density polyethylene), Even Span or Gable Type (two roof slopes, height at gutter 3 meters and up to 4.5 meter at centre, constructed by round or square steel tubes covered with glass, polycarbonate or polyethylene), Quonset Type(roof semi-round, covering material could be a polyethylene or polycarbonate panel). The cost of the green house depends upon various factors such as the type of material used for construction of the green house, misting system, exhaust fans, cooling pads, humidity chambers and racks.

3.2 Equipments

As earlier described that tissue culture laboratory have various working areas for different purpose, such as media preparation and sterilization room, washing room, transfer room, culture room, store room, acclimatization room etc. These area have variety of equipment/apparatus for tissue culture operation. The quantity and type of equipment will be depend on budget as well as installed production efficiency. In general laboratories following equipments are needed to operate tissue culture experiments:-

3.2.1 Glasswares and plasticwares

Different types of labwares, glasswares or plasticwares have been used in tissue culture laboratory for various purpose .Mainly labwares are utilized in the tissue culture experiments (tissue, organ, protoplast, micro propagation etc.), preparation of media, sterilization and storage (media, chemicals, distilled water etc.). Test tubes, small capacity flasks, petri-plates, glass bottles have been used in tissue culture practice. Whereas, large capacity flasks, measuring cylinders, plastic cans etc are required for preparation of media, sterilization and storage purpose. The nature of glassware/labwares used in tissue culture depends on the type of experiment, which can be done and also on the preference and convenience of the researchers. In most of tissue culture experiment glass test tubes have been used. Where as wide mouth glass bottles have been used in micro propagation experiments. These glasswares may be made up of pyrex or borosilicate, which are heat resistant and less susceptible for breakage and scratching. In some cases soda glass can be used, but repeated use can cause toxic effect on some cultures. In recent time glasswares are largely replaced by autoclavable plasticwares. Disposable plasticwares (jars, petri-plates, measuring cylinder etc) also available in the market. The suitable closures are required for culture vials, flasks, which prevent contamination and loss of moisture content. Cotton plugs or aluminium foils are used for culture tubes or flasks in traditional way, but there are time consuming and inconvenient. Alternative plastic or metallic cap closure can be used.

3.2.2 Water distillation unit

Sterile water is used in almost all processes in the laboratory, but mainly in media preparation and sterilization. In the teaching laboratory de-ionized water may be used for media preparation. While in the research or commercial scale laboratories milli-Q water purification unit or water distillation unit must be required. Milli-Q water free from microorganisms, particle traces, colloids, ions, organic impurities. De-ionization unit work on the principle of ion –exchange methodology. So de-ionized water free from ionic impurities. Whereas, water-distillation unit work on the principle of boiling of water and then condensation of vaporized water in separate container. This water free from suspended particles, colloids, and microbes etc. Laboratory can installed small or large distillation or deionization units according to their water demands.

3.2.3 Weighing balances

High quality weighing balances are essential for a tissue culture laboratory. A weighing balance is used to measure the mass or weight of the all kinds of objects. Multiple weighing balance are available in the laboratory with various range weighing capacity as; top pan balance, precision balance, analytical balance and moisture analysers are required in general tissue culture laboratory. Usually the top pan balance is used for measuring larger quantities (above 10gm). Precision balance are used to measure the weight of object at readability > = 0.001gm. Whereas analytical balance can used to measure the weight with 0.1mg to above range. Moisture analyser are used for analysis the mass of the object. The analytical balance is more precise than precision balance, and have higher readability and lower capacity in comparison to precision balance.

3.2.4 pH meter

pH meter used to determine the acidity or basicity of solutions . The pH value depends on the H+ ion concentration in the solution. The value of pH meter is 0 to 14 range. pH value 7 denote neutral property of solutions,

whereas less than 7 or more than 7 show acidic and basic property of solutions respectively. pH meter is a electrical device, which contain ph probe with two electrodes. One is sensor electrode and other is reference electrode. pH probe passes electrical signals to the pH meter, which display the pH value of solutions.

3.2.5 Autoclave

This equipment is a vital part of a tissue culture laboratory and used to sterilize the media, water, glassware or utensils. It works on the principle of steam sterilization. Moist heat kills the microorganism by disrupting their metabolic components like proteins, enzymes and lipids. Temperature is directly related to the sterilization whereas pressure is indirectly related. The temperature 121°C-132 °C at 15psi (106 kpa) pressure is maintained for 15 to 30 minutes depending upon the volume of flask to be autoclaved. Sterilization temperature varies according to the volume of culture media and number of labwares or items to be autoclaved. Autoclave may be small or large size with 5 to several hundred liters holding capacity can be installed based on the experimental requirement. Autoclave is made up of following components or parts:- Autoclave is a hollow cylindrical body which consist of inner chamber and an outer jacket. Body of autoclave may be made by aluminium, stainless steel or gun metal. Lid (to ensure an air tight closure), gasket (to provide leak proof contact between main body and lid), pressure gauge (indicate pressure which is generated during sterilization) pressure releasing valve (functioning same as pressure cooker whistle), radial wing nut locking system, pedal lift, timer and indicator etc. Steam generator or electrical heater is present in bottom of the inner chamber, it generate heat during the sterilization. These heating elements immersed in the water. (See Fig.6 Outer and inner view of autoclave.) During the sterilization process heating element heat the water and than steam is generate in the inner chamber. This steam de-contaminate the media or items by disrupting microbial metabolic components. When pressure of the autoclave zero and temperature below from 50%, then autoclave should be open. Autoclave used in the laboratory may be pressure cooker type,

horizontal or vertical type. If the autoclave facility not available in the laboratory then pressure cooker can be used for this purpose. (further details can be seen in another chapter)

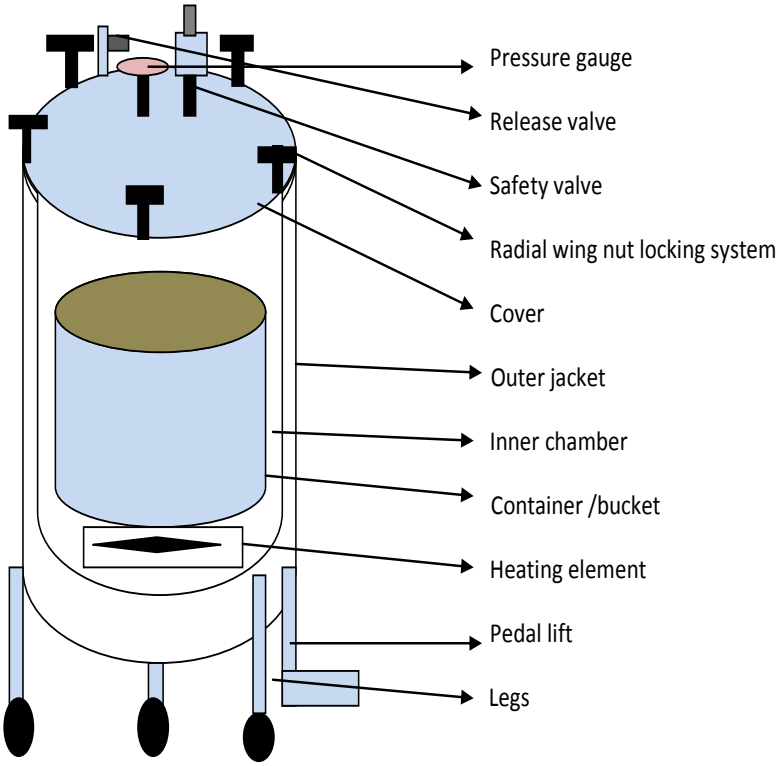

FIG.:-LINEAR DIAGRAM OF AUTOCLAVE

Figure 6: Outer and inner view of autoclave

3.2.6 Laminar Air – Flow Bench (LAF)

To achieve aseptic environment, laminar air flow cabinet are used for the culture transfer in most of laboratories. A laminar air flow bench is sufficient tool for all the activities of sterile transfers. Basically, it is a clean bench with protection of UV light and filters (pre filter and HEPA filter) that assures the contaminant free work area at the time of inoculation, transfer and subculture. The laminar air flow is usually made of stainless steel with no gaps and consists of air tight chamber, pre-filter, HEPA filter, blowers, work bench, pressure manometer, shutters, operating switches of blower

and UV light. HEPA filters have very small pore size (0.3μm) with high efficiency. (See Fig.4 Schematic diagram of LAF cabinet.) UV light (uv-c germicidal) switching on before 30 min to start the work and later switch on the air blow. During the laminar air flow functioning, blow air first passes through the pre-filter (in which large particles removed) and then through a fine filter. The fine filter also known as HEPA (high efficiency particulate air) filter. This filter removes the particles larger than 0.3 μm and free from microbial contaminants .This ultra clean air passes through the working area. The air velocity of ultra clean air is approximate 27±3 m/min, which is adequate for prevention of contamination outside to inside by the worker. Other contaminants such as hairs, salts, flaks also can not entered inside the laminar. So the fine filter provides the contamination free environment for transfer practice. The air flow does not interrupt the work of Bunsen burner. Depending on the air flow direction laminar air flow can be divided into horizontal or vertical type. In temperate areas laminar air flow cabinet are kept in ordinary laboratory. While in tropical and sub-tropical areas the cabinet should be fitted in separate room with double door facility, which maintain aseptic environment and also prevent from atmospheric dust.

3.2.7 Culture shelves or culture racks

Specially designed racks mainly constructed by glass or rigid wire mesh should be essential to store cultures. Each shelf has separate fluorescent tubes. Insulation should be maintained between lamps and shelf to ensure more even temperature around the cultures.

3.2.8 Air-conditioners (AC)

The maintenance of desired temperatures in growth room as well as inoculation room would be possible by the AC with efficient cooling capacity. The working principle of air conditioner is collect hot air at particular space and than release cold air at the same space.

3.2.9 Filter sterilization

Sterilization of thermolabile solutions carried out by membrane filtration, which have bacterial proof membrane with pore size 0.45 µm or less. The membrane fitted into appropriate size filter holder and then autoclave with aluminium foil covering. The 121˚C sterilization temperature is optimum for filter, not to be exceed. A graduate syringe (singe time used) filled with solution, fixed at one end of the filter and pushed through membrane. Filtrate collected from the other end into separate pre sterilized beaker or jar. Large filtration units also available for sterilization. The solution should be clarified by no.3 pore size sintered glass filter prior to filter sterilization. This is useful to reduced the blockage of membrane filter pores.

3.2.10 Hot air oven

Hot air oven is a device that used for dry heat sterilization, it does not require water. This is convenient for glasswares (test tubes, flasks, petri plates etc), oil containing material, powders (e.g. starch), metallic apparatus (forceps, blades, scissors, scalpels etc). Plastic or rubber material not to be sterilized in a hot air oven. Extremely higher temperature over several time require for sterilization to destroy microorganisms or spores. According to temperature variation, the sterilization time is also vary.

Hot air oven made up of two parts:-one is mechanical part and other one is electrical part. The mechanical part has been divided into the outer shield, fiber glass, the chamber and the shelves. Whereas electrical part mainly consist of the power supply, heating element, thermostat, timer, fuses, temperature indicator etc.

3.2.11. Other equipments

A variety of equipments (such as microscope, microwave, glass bead sterilizer, centrifuge, pipettes, hot plate/stirrer, lab washers, and computer system etc.) are also used for laboratory purpose.

3.3 Chemical requirements

Different types of nutritive components are needed to prepare culture media for efficient growth (see details in chapter 3). Different plants or even different parts of the same plant may have different nutritional requirement. So a single media cannot be suggested for different tissue or organ culture. However, in most of tissue culture experiments murashige and skoogs medium (MS medium) composition is used, where as potato dextrose agar(PDA medium)is used for fungus cultivation. The basic requirements for general tissue culture practice are described below. These components mainly described in to four major categories, such as inorganic nutrients, organic nutrients, growth hormones and gelling agents.

3.3.1 Inorganic nutrients

Inorganic nutrients are very important for plant growth and development. The inorganic components consist of micro nutrients (Fe,Mn,Cu,Zn,B,Mo,Co) and macro nutrients (N,P,K,S,Ca,Mg). Macro nutrients are those elements, which are required in large quantities (1000mg/kg of dry weight), whereas micronutrients are needed in small amount or trace quantities (≤100mg/kg of dry weight). The essential elements with their forms and functions are describing in table no.2

Type of the element	Name of the elements	Available forms	Absorpt-ion forms	Functions
Macro elements	Nitrogen(N)	KNO_3 NH_4NO_3	NO_3^- NO_2^-	Constituent of amino acids ,proteins ,nucleotides, co-enzymes, nucleic acid, amides

		$NaNO_3$	NH_4^+	
	Phosphorus(P)	KH_2PO_4	$H_2PO_4^-$ HPO_4^-	Involve in cell division, ATP, AMP, GTP, NADP, Nucleotide, Phospholipid synthesis
	Sulfur (S)	$MgSO_4..7H_2O$ K_2SO_4	SO_4^-	Constituents of cysteine, cystine ,methionine
	Calcium(Ca)	$CaCl_2.H_2O$	Ca^{+2}	Cell wall synthesis, metabolic regulation
	Potassium(K)	KNO_3 KCl	K^+	Enzyme activation, ionic balance ,osmo regulation ,cell division
	Magnesium(Mg	$MgSO_4.7H_2O$	Mg^{+2}	Constituent of chlorophyll molecule
Micro elements	Iron(Fe)	$FeSO_4.7H_2O$ $FeSO_4.5H_2O$ $FeCl_3.6H_2O$	Fe^{+3}	Involve in photosynthesis, nitrogen fixation and respiration
	Manganese(Mn)	$MnSO_4.4H_2O$	Mn^{+2}	Activation of some dehydrogenases, decarboxylases, kinases, oxidases, photosynthetic O_2 evolution
	Zinc(Zn)	$ZnSO_4.7H_2O$ $ZnSO_4.4H_2O$ $ZnNa_2.EDTA$	Zn^{+2}	Constituent of enzymes

	Molybdenum (Mo)	MoO_3	Mo^{3++} Mo^{2++}	Oxidation-reduction reaction
	Copper(Cu)	$CuSO_4.5H_2O$	Cu^{+2}	Redox reaction ,photosynthesis,respiration
	Boron(B)	H_3BO_3	BO_3^-	Constituents of cell wall,cell elongation ,nucleic acid metabolism
	Cobalt (Co)	$CoCl.6H_2O$ $CoSO_4.7H_2O$	Co^{+2}	Nitrogen fixation
	Chlorine (Cl)	$CaCl_2$ $CaCl_2.2H_2O$	Cl^-	Osmo regulation

Table no.2 Essential elements with their forms and functions.

3.3.2 Organic nutrients

The organic components consists of mainly vitamins, amino acids, carbon sources and gelling agent. The major essential organic components with their respective function are presented in Table. no 3.

Types of chemical components	Name of the components	Probable functions
Vitamins	Thiamine (B1), Pyridoxine (B6), Nicotinic Acid (B20), Biotin, Folic acid, Riboflavin Vitamin-C,D,E etc.	Promote cell growth and development
Amino acids	As glycine, arginine	May directly utilized by plant cell or serve as

		nitrogen source.
Carbon sources	Sucrose, glucose, fructose, maltose, Lactose, galactose, manose etc	Source of carbon
Undefined supplements	Casein, yeast extract, corn milk, coconut milk, malt extract, tomato juice	Promote callus or organ growth.
Plant growth hormones	Auxins : Indole -3- acitic acid(IAA) Indole- 3 –butyric acid(IBA) Naphthalene acitic acid(NAA) 2,4-dichlorophenoxy acitic acid(2,4-D)	Cell division Callus formation and growth Rooting (at high auxin conc.) Shooting(at low con.)
	Cytokinins : Kinetin , Zeatin, Benzyladenine (BAP) 2-isopentenyladenine(2ip)	Cell division Root formation inhibition Adventitious shooting Callus formation and growth
	Gibberellins	Shoot elongation Dormancy

	Others : Ethylene, abscisic acid, jasmonic acid	Somatic embryogenesis Shooting, rooting Enhance meristem formation etc.

Table no 3. Major essential organic components with their respective function.

3.3.3 Growth hormones:

Plant hormones are organic compounds, which are capable to change growth or morphology of the plant at very low concentration. Mainly auxins, cytokinins, gibberellins, are used in tissue culture. Other hormones as abscisic acid, ethylene, jasmonic acid etc. also used in some specific experiments. The plant growth hormones and their functions are described in table no.3.

3.3.4 Gelling agents

Gelling agents are used for preparation of the solid or semi-solid culture medium. Most of gelling agents (agar, agarose, gelrite etc) are obtained from biological products .Like agar is obtained from red algae.

3.4 Other requirement

Some other requirement such as power supply, water supply, cleaning and drying facilities should be needed for operation of tissue culture laboratory. Automation of all or some of the infrastructure should reduce the overall production cost and enhance the production efficacy of the laboratory.

4. Safety Consideration

In tissue culture laboratory, hazards could be associated with instruments and their handling, sterilization techniques, explants and experimental

methodology which may be harmful or change the regular biological functions. Hazards may be of mechanic, chemical, ionic or biological origin. Regardless of their hazards researcher/culturist do work with proper safety and adequate personal protection equipments. A laboratory training can be carried out before tissue culture operations for safely handling. Some safety consideration approaches to reduce these issues are discussed below.

1. Eating, smoking, drinking should be avoided.

2. Noxious chemicals such as organic solvents (i.e. alcohol, chloroform, halogens, mutagenic chemicals, mercury compounds etc.) should be handled with proper precautions.

3. Fractured glassware and knife, blades or needles must be disposed of differently labelled dustbin.

4. Fire extinguishers and first aid box must be provided in the laboratory.

5. Dispose of all liquid or solid waste after each experiment with bleach and autoclave.

6. Washing of hands after handling culture and before leaving lab.

7. Decontaminate work surface with disinfectant (before and after).

Some of the signs can be placed properly in the laboratory to avoid any kind of accidents (Fig 7)

Biohazard warning sign	Food and drinks are prohibited

 Corrosive materials include strong acids and bases	 Material is flammable
 Put your shoes outside	
Fig 7: Plant tissue culture Laboratory safety signs	

The work has been cited from following references, further reading can be done from these sources:

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